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NUTRITION REVIEWS

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SALT

It is known that diets low in sodium may be beneficial in the treatment of hypertension, and are essential in the management of congestive heart failure. In the light of this knowledge there has been renewed interest in the role of sodium chloride in physiologic processes in health and disease.

There is ample evidence that ancient civilized man was acquainted with salt. However, as other territories were opened, it was found that uncivilized people in widely scattered areas were likewise using salt. It has been suggested that salt was a primeval discovery of man (M. Graubard, "Man's Food," *Macmillan, New York (1943)*). Whether primitive men experimented by tasting deposits of salt, or were first led to it by animals at salt licks, is unknown, but once the condimental value of the substance was realized, the demands for it became legion.

Early Manufacture and Traffic in Salt: Attempts to manufacture salt in quantity appear to be as old as recorded history. The Chinese (ca. 2700 B.C.) were known to have obtained salt by solar evaporation of saline or by rock salt mining. Salt was also prepared by boiling kelp over fires made of salt weeds (L. G. M. Baas-Becking, *Scientific Monthly* **32,** *434 (1931)*). Sea salt manufacture in the Mediterranean area seems to have originated with the Phoenicians. They shipped it in cargo for trade. In Greece the virtues of salt were extolled. Slaves were bought and sold with it; the good slave was "worth his weight in salt." Roman salterns were located near Ostia and the Dnieper River pans were also known to yield good salt. The most famous salt came from Citium, on the island of Cyprus. For the purpose of salt-making, the solar evaporation of sea water in masonry vats, or the boiling of sea water or natural brines became a highly developed art. Many red salt deposits and lakes were known in the Middle East.* The red salt garnered from these deposits appeared to be no less marketable than the whiter salt from the sea.

Although the want for salt was great, the yield from the manufacturies was so scant that supply was unable to cope with demand. The crudeness of transportation further added to short marketable supplies. In rattling carts and clumsy caravans the precious mineral was taken inland from the salterns, traded for rich cargoes of other materials, and the caravans clattered to the shores again for another supply of salt. The *Via Salaria* of Rome was the chief trade route from the city to the sea and it became, in time, a well-guarded artery for imports of salt. Salt was frequently valued as money and often served as a medium for salaries (L., *salaria*) (H. W. Van Loon, *Natural History* **39,** *79 (1937)*).

During the Roman conquest of northern Europe the search for salt continued. People of Gallic and Germanic provinces were found obtaining salt by pouring brine over burning wood. After the conquest of England, the Britons, under their new overlords, worked their native brine springs to recover salt by boiling off the water. Germanic tribes fought wars over the possession of salt streams (Baas-Becking; Van Loon, *loc. cit.*)

Symbolism of Salt; Taking of Covenant: In Biblical writ there are numerous references to the use of salt as a necessity, as well as in symbolism. In ancient Hebrew lore, salt was capable of preserving from corruption. At this time the cementing of agreements and

* Concentrated salt brines harbor a multitude of organisms. Reddish bacteria (the "codfish bacteria") and chlorophyll-containing plants are now known to be the chief causes of red waters (Baas-Becking, *loc. cit.*)

the obligation of men to duty were achieved through covenants described in both the Old and New Testaments. The breaking of covenant was tantamount to crime. The origin of covenanting is vague, but the covenant taken with salt was undoubtedly the most binding (H. C. Trumbull, "The Covenant of Salt," *Scribner's, New York (1899)*). The binding of salt covenant was no less exacting for the Arabs, nor was it less respected. A man of particular worth "had salt." Salt prevented decay; by analogy, it preserved flesh as well as soul from corruption.

Conversely, salt also became symbolic of destruction. This concept perhaps arose in the Middle Eastern deserts by the mistaken drinking from saline pools. The story of Lot's wife turned to a pillar of salt has become familiar. Sowing a land with salt rendered it permanently useless and desolate.

Later History of Salt: With the expansion of populations and of trade in the Renaissance, the hunger for salt continued. As in the spice trade, a consignment of salt frequently changed hands so many times its actual origin was lost in the tracery of repeated barter. Wars and political upheavals often disrupted the trade, but only temporarily, for the demand was great and the salt routes were quickly reopened as the wars subsided. In this period it was a privilege to own the mineral. The tables of royal halls usually included an imposing saltcellar for the exalted substance. At these tables the more important persons were placed to "sit above the salt"; less prominent guests were seated lower than the saltcellar. The inequity of supply and demand also fostered salt taxes, occasionally of crushing severity. Military organizations likewise clamored for salt. It was assumed that Napoleon's army was decimated in its retreat from Moscow in part because of the lack of salt to sustain the men in the hardships of winter. Navigators required it for food preservation during long voyages at sea.

The passage of history has repeatedly shown that the hunger for salt drove men to fight, bargain, migrate, trade. The American colonists were not without their periods of salt hunger. Salt was originally obtained only by costly importation. In a few years, however, salt was being manufactured by the evaporation or boiling of sea water in Massachusetts, Long Island, on the Jersey shores and elsewhere. Enough salt was made so that imports could be greatly reduced. Salt manufacturing suffered during the Revolutionary War, for the salt plants were singled out by the enemy for complete destruction. After the war the inland brine springs were quickly turned to use. The salt springs at Lake Onondaga in New York were sought out, and thereafter, for many years, the Onondaga area, including Syracuse, was the chief salt manufacturing center of the country. Brine wells were also found along the Kanawha River and in the hills of southern Virginia where the community of Saltville was established. These salt workings became of major importance in turn. It is interesting that the Confederate loss of Saltville in the Civil War may have hastened the crumbling of Confederate resistance (G. L. Eskew, "Salt, The Fifth Element," *J. G. Ferguson, Chicago (1948)*).

The opening of additional salt deposits and refinements in salt mining and manufacture have been a story of industrial progress over the period of a century (Eskew, *loc. cit.*).

Salt Hunger; Salt and Flavor: Years of observation have shown that herbivorous animals frequent salt licks; on the other hand, carnivorous animals show little interest in supplementary salt intake. That grazing animals require additional sodium chloride was borne out by one observation (S. M. Babcock, *Wisconsin Agr. Exp. Sta., Annual Report, p. 129 (1905)*) that lactating cattle deprived of salt will weaken, lose weight and become haggard. More recently it has been shown that experimental animals deprived of dietary salt, or those in which adrenalectomy has produced a chronic salt

deficit, will naturally choose enough sodium chloride to render themselves comfortable or to keep alive (C. P. Richter, *Harvey Lectures* **38,** *63 (1942/43)*). When such physiologic irregularities occur, the question arises as to whether it is experience, the want of a feeling of well-being, or taste which determines the natural selection of salt supplement. The evidence indicates that *taste* may be important in this selection. After the gustatory nerves were severed in rats, the adrenalectomized animals no longer increased their salt intake and they died with marked sodium deficits. It is of interest that some patients with Addison's disease express a "craving" for salt and will add it in large quantities to their food. Furthermore, people who subsist largely on vegetable matter often describe an intensified desire for salt, and those living on meat desire less salt. Such observations have led to the suggestion that *taste* and *flavor* may be significant regulatory mechanisms relating to the natural correction of deficiencies of specific minerals, proteins or other essential materials. The consequences of acute and chronic sodium chloride deficiency in man are well known and need not be repeated here. But it is perhaps something more than fortuitous that the addition of salt to food sharpens natural flavors and, therefore, may tend to rectify dietary deficiency by adjustments of appetite.

The physiologic mechanisms by which salt influences flavors are obscure. It is generally held that flavor is a combination of gustatory and olfactory sensations. The four primary taste stimuli (sweet, salt, sour and bitter) are therefore modified by what is simultaneously smelled, and the total of these stimuli blend to cause flavor. It has been observed that one prime effect of sodium chloride as a seasoning agent is to suppress "rawness" and sourness as the first step in the correction of undesirable tastes, and the flavor may then be controlled by the further addition of sugar, pepper, or other seasoning (S. E. Cairncross, "Flavor and Acceptability of Monosodium Glutamate," *p. 32, Proc. Sympos., Quartermaster Food & Container Inst. for the Armed Forces and Assoc. Food & Container Inst., Chicago (1948)*). Proper salt seasoning increases the total volume of flavor, suppressing some notes of natural flavor without destroying identity, and accentuating desirable flavors. Monosodium glutamate, an adjunctive seasoning (with salt) is also capable of modifying rawness and other undesirable tastes. This effect of monosodium glutamate may be mediated through all of the taste buds because sourness, sweetness, saltiness and bitterness are recognizable in this substance (E. C. Crocker, *Ibid., p. 25*). These data raise the question of how an increase in the salinity of foodstuffs may influence odor during mastication. Since odors are more sharply perceived in substances with high vapor densities (large molecules), it may be suggested that sodium chloride tends to mobilize odorous materials in the food which then escape and diffuse to the olfactory mucosa. If olfaction is sufficiently intensified in this manner, there may be marked augmentation of flavor. The addition of sugar or acid to food may also alter flavor by the release of odorous compounds. It is realized that these suggestions are empiric; the mechanisms by which salt imparts relish to certain foods are little understood.

In modern clinical practice the use of low sodium diets is considered essential in the treatment of congestive heart failure. It has been repeatedly demonstrated that failure of cardiac output and the gradual reduction of renal blood flow, together with possible extrarenal hormonal factors, induce retention of sodium by the kidneys, and hence retention of body water, so as to increase the circulating blood volume. The increase in circulatory load enhances the elevation of venous pressure and the formation of peripheral edema (J. O. Davis and J. R. Smith, *Am. J. Med.* **3,** *704 (1947)*; H. A.

Schroeder, *Circulation* **1,** *481(1950)*). Therefore, with supportive treatment of the myocardium by rest and digitalis, adequate restriction of salt (*i.e.*, 0.5 to 1.0 g. per day) seldom fails to prevent the retention of water unless failure is progressive and intractable. Further salt elimination may be sought by the administration of mercurial diuretics. It has also been shown (Schroeder, M. L. Goodman, P. H. Futcher, and M. Hunter, *J. Am. Med. Assn.* **140,** *458 (1949)*) that some patients with hypertension may respond with a significant fall of blood pressure to drastic reductions in salt intake. The clinical observations in such cases suggest alterations in adrenal cortical function in relation to sodium metabolism. However, the reduction of salt intake in hypertension is usually ineffective when the condition is advanced. A low sodium diet is disagreeable or even intolerable to some individuals, and they refuse to take it without supplemental salt. The addition of commercial salt-substitutes to improve food flavor has often induced patients to accept the necessary therapeutic regimen.

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INFANTILE PELLAGRA

Since the Children's Hospital of Mexico was opened about eight years ago, many cases of pellagra in children have been observed. A summary of the findings and conclusions to date from studies of this abundant clinical material has been reported by J. Muñoz Turnbull (*Gaceta Médica de México* **81,** *282 (1951)*).

Approximately 1.9 per cent of all cases admitted to the hospital are classified as pellagra. During the summer months the incidence may rise to 20 per cent of all beds assigned to children of preschool age. As discussed below, the high incidence during the hot weather is believed to be directly related to the high incidence of diarrhea at this time. An age distribution curve presented shows that the incidence rises very rapidly between 1 and 2 years and reaches a maximum in 2 year olds. The number of 3 year olds has been approximately half that of 2 year olds and the incidence gradually falls with age until few cases are seen over 8 or 9 years of age, although some cases have been observed at all ages up to 14 years, which is apparently the eldest group admitted to the Children's Hospital.

The dietary pattern in *all* cases is similar. It consists of "atoles" without milk (thin corn gruels), beans and corn, and sugar. More recently some other cereals have been added, especially tortillas and occasionally rice. Fruits are rare. Milk is sometimes reported, but this consists of small quantities once or twice a week mixed with a cup of coffee or infusion of orange leaves. The diet is thus extremely low in milk products, meats, greens and eggs.

The semantic problem of whether the disease is or is not pellagra is raised again with the opposite conclusion to that which most investigators have reached. While many have concluded that the disease in children is not pellagra because it does not respond to niacin therapy alone, Muñoz Turnbull and collaborators conclude that pellagra is a multiple deficiency disease. Their argument runs as follows: The children are deficient in protein as evidenced by low serum protein levels in 77 per cent of the cases and low serum albumin levels in 86 per cent. The serum globulin was high in 32 per cent. The authors cite the well-known studies of Goldberger and the tryptophan-

niacin relationship as evidence of protein deficiency in pellagra. They also recall the studies of P. Handler (*Proc. Soc. Exp. Biol. Med.* **52,** *263 (1943)*) that blacktongue in dogs is not produced by simple niacin deficiency.

These children are believed to be lacking niacin, however, as evidenced by the dermatitis which begins upon the dorsal parts of the feet and hands and later extends over the body, and by the mucosal lesions. The latter are described as a stomatitis and a glossitis characterized particularly by the slick brilliant red tongue and atrophy of the lingual papillae. In older children psychic disturbances—confusion, disorientation, and fear—which respond to niacin therapy are seen. Findings of cheilosis, acne of the nose and surrounding area, typical dermatitis, conjunctivitis and circumcorneal congestion, and burning sensation of the eyes are interpreted as evidence of clear-cut riboflavin deficiency. Ninety-five per cent of the pellagrous children were found to show electrocardiographic changes identical to those seen in beriberi (J. de la Torre, S. Berber, and L. Duran, *Boletin Médico del Hospital Infantil de México* **6,** *317 (1949)*). Ninety-nine per cent have been reported to have electrical changes in the muscles similar to those seen in beriberi (de la Torre and A. Tohen Zamurio, *Ibid.* **5,** *162 (1948)*). In addition, the urinary thiamine excretion is very low, there is hypotonia of the muscles and in some cases loss of the patellar reflex.

The author concludes therefore that these children are suffering from deficiencies of protein, niacin, riboflavin and thiamine, with the probability that other vitamins which have as yet not been studied are also low.

Diarrhea is believed to be an etiologic factor rather than a symptom of pellagra. As has been indicated, most of the pellagra occurs in the summer and it is usually preceded by diarrhea for several weeks or months. Most of the diarrheas can be cured by proper therapy within a few days after admittance while the other symptoms require several weeks or longer to disappear. The consumption of contaminated foods by the susceptible infant shortly after weaning is thus considered a primary etiologic factor in the high incidence in 2 year olds. Increased resistance to the diarrheas rather than any great improvement in food intakes is thought to account for the lower incidence with increasing age.

Liver biopsies on 51 children on admission showed 100 per cent incidence of fatty infiltration similar to that described by T. Gillman and J. Gillman (*J. Am. Med. Assn.* **129,** *12 (1945)*). Although the severity varied, it is stated that in some cases it was difficult to distinguish the liver biopsy specimen from a section of perirenal fat. Bromsulfalein tests were uniformly negative, however, and prothrombin time was normal. Serum cholesterol esters are reported as low in 64 per cent, thymol turbidity as positive in 73 per cent, and cephalin as positive in 60 per cent. Liver biopsies taken after a month of treatment showed improvement or cures in 86 per cent and the abnormal liver function tests improved in the majority of cases.

Nearly all of the children with pellagra showed some evidence of lung involvement. Radiologically the lesions are similar to and have often been reported as tuberculosis. The authors were unable to demonstrate tuberculosis in most of the cases by means of the Mantoux test, sedimentation rates, and examinations of gastric washings for the Koch bacillus. Fever is generally lacking and the lesions regress slowly with diet therapy. Such regression is always much slower than the other symptoms of the disease. On the basis of these studies it is concluded that the radiologic lesions of the lungs are actually the result of the dietary deficiency.

Little is said of the preferred dietary treatment other than that diets very rich in proteins, 4 to 5 g. per kilogram of body weight per day, supplemented with vitamins

are recommended. The over-all mortality has been 26.2 per cent in pellagrous cases and it is thought that this will decrease in the future with the better knowledge now available.

The present report leaves considerable information to be desired. Particularly, the statistical data on the occurrence of the various lesions are lacking. It is believed apparently that the disease is the same as that which has been studied extensively by the Gillmans in South Africa. Little is said of the incidence of edema, other than that it occurs, while this is a major symptom in South Africa (J. Gillman and T. Gillman, "Perspectives in Human Malnutrition," *Grune and Stratton, New York (1951)*). Although some bone marrow studies are presented, few data on the blood picture are given. Critical evidence for the importance of the various nutrients cited above is lacking. Presumably such data will be made available in the future.

Whether the disease is identical with kwashiorkor (*Nutrition Reviews* **10,** *261, 297 (1952)*) remains obscure, but the similarity is obvious and protein deficiency must be a major factor in both. In this regard it is of interest that whereas the Gillmans have concluded that niacin therapy was contraindicated, the addition of large amounts of niacin to the diets used in Mexico, 75 to 150 mg. per day, have no untoward effects and are believed to have been beneficial.

NITROGEN METABOLISM IN CUSHING'S SYNDROME

The clinical entity known as Cushing's syndrome is associated with and probably due to hyperfunction of the adrenal cortex. This is apparently idiopathic, due to tumor of the pituitary presumably releasing larger than normal amounts of adrenocorticotropic hormone (ACTH), or due to tumor of the adrenal cortex itself, producing excessive quantities of cortical hormones. Clinical findings are the so-called "buffalo obesity," but with wasting of muscle mass, hypertension, osteoporosis, and, in women, masculinization. The striking feature of muscle wasting was at first explained on the basis of observed negative nitrogen balances. This explanation became less satisfactory when some patients with typical muscular wasting were observed to be in nitrogen equilibrium. This and other circumstances led F. Albright (*Harvey Lectures* **38,** *123 (1942/43)*) to suggest that instead of continuous protein catabolism in Cushing's syndrome there was in reality an antianabolism.

Further evidence in favor of an antianabolism to account for part of the defect in nitrogen metabolism in Cushing's syndrome has been presented by W. Parson, K. R. Crispell, and A. Ebbert (*J. Clin. Invest.* **31,** *548 (1952)*) using N^{15}-tagged glycine as a tracer. The subjects were maintained on a constant and presumably adequate diet. A measured amount of glycine was given daily in addition to that in the diet. N^{15}-glycine was substituted for the normal N^{14}-glycine on the "test day." Urines were collected at suitable periods thereafter (usually for forty-eight hours) and were analyzed for N^{15}. Eight studies in 7 healthy volunteers gave relatively reproducible curves of N^{15} excretion which was expressed as per cent of that ingested. These normal subjects were in nitrogen equilibrium.

Patients with Cushing's syndrome demonstrated a marked increase in the percentage of isotopic nitrogen excreted within these forty-eight hours as compared to the normal individuals, but usually not with the appearance of negative nitrogen balance. When one patient with Cushing's syndrome was given testosterone propionate, the rate of

excretion of isotopic nitrogen, although slightly greater than normal in the first eighteen hours, was thereafter within the normal range. Likewise, one patient with Cushing's syndrome treated with x-ray had a definite decrease in the percentage of isotopic nitrogen excreted.

Finally, on three occasions the administration of ACTH induced an increase in the excretion of isotopic nitrogen in 2 normal individuals, although in only two of these three instances were negative nitrogen balances observed.

The authors believe with Albright that the action of the adrenal hormones was largely antianabolic, since increased isotopic nitrogen excretion occurred whether or not negative nitrogen balance existed. However, x-ray and testosterone propionate therapy did induce a coincidence of positive nitrogen balance and reduction in isotopic nitrogen excretion.

THE LIPIDS OF SEBUM

The skin, in addition to providing a protective covering for the body, is concerned in many metabolic reactions involving a variety of nutrients. The secretions of the sweat glands form a pathway whereby water, water-soluble vitamins, electrolytes and metabolic end products are lost to the body. The skin is the site of conversion of provitamin D to vitamin D. In addition, deficiencies of certain essential nutrients include manifestations in the skin. This is true of vitamin A, niacin, pyridoxine and essential fatty acid deficiencies.

Sebum, the secretion of the sebaceous gland of the skin, is characteristically oily. There is little information available which would describe the possible roles of the skin as an organ involved in lipid metabolism. An avenue of approach to this problem would seem to reside in the study of sebum. Investigators at the Medical College of St. Bartholomew's Hospital in London have attempted to characterize the constituents of surface skin fat from the human forearm (R. M. B. MacKenna, V. R. Wheatley, and A. Wormall, *J. Invest. Dermat.* **15,** *33 (1950)*). In these studies sebum was obtained by immersing the forearm in a cylinder containing acetone for three minutes. The process was then repeated with the opposite forearm. The acetone extract was taken to dryness and re-extracted with chloroform. Under standard conditions the quantity of lipid obtained from the sebum of a normal adult was relatively constant, about 0.1 g. in three hours.

Sebum, extracted and prepared by this method, was found to contain an average of 29 per cent free fatty acid, 36 per cent combined fatty acid present in glycerides, waxes, and other esters, and 32 per cent of an unsaponifiable fraction. Of particular interest to the investigators were materials included in the unsaponifiable fraction. A variety of hydrocarbons, cholesterol and other unidentified substances were included. A single hydrocarbon which was demonstrated to be squalene comprised about 16 per cent of the unsaponifiable matter or 5 per cent of the extracted sebum. Squalene is a thirty-carbon compound containing six double bonds. It has been found previously in liver oils of shark and other fish, as well as in yeast. Interest would center on whether or not squalene has some specific function in the metabolism of skin or whether it appears in sebum simply as an end product of metabolism.

The earlier observations of these investigators have been extended and provide the material for a recent publication (R. M. B. MacKenna, V. R. Wheatley, and A. Wormall, *Biochem. J.* **52,** *161 (1952)*). The observations described pertain most im-

portantly to a further study of the unsaponifiable material of sebum and the fat-soluble vitamins in this secretion of the skin. Sebum was collected as described in the earlier paper. That extracted from the forearms of from 15 to 82 normal subjects was combined to make available larger quantities of material for analysis. After re-extraction with chloroform the sebum was saponified and various fractions were derived by chromatography using alumina columns with petroleum ether and chloroform as solvents. Iodine numbers, acetyl numbers, melting points and molecular weights of the various fractions were determined by standard methods and the data recorded.

The results of these studies indicate that the unsaponifiable component of sebum includes a variety of substances. Those appearing in greatest quantity include hydrocarbons which account for 30 to 46 per cent of the total unsaponifiable fraction; normal-chain aliphatic alcohols, 14 to 20 per cent; and cholesterol, 14 to 19 per cent. Other compounds derived by chromatography were shown to be oxidation products of squalene, or were not totally identified.

The authors consider the possible significance of squalene as an important constituent of sebum. They point out that squalene accounts for 30 to 40 per cent of the hydrocarbon fraction of the unsaponifiable material. The remainder of this fraction was found to be mainly normal paraffins. The authors state that these hydrocarbons may simply be metabolically useless end products, but consider the other point of view that might be offered, that is, that these compounds have certain as yet undescribed functions in skin metabolism. It is of interest that squalene has been found in other sebum-like materials from human subjects, in the contents of dermoid cysts, the natural oils of hair, and cerumen (H. Sobel, *J. Invest. Dermat.* **13,** *333 (1949)*). The functions of squalene and other hydrocarbons as components of the secretions of the sebaceous glands are not readily apparent. It is of interest, however, that these compounds derive as products of the metabolism of human skin.

Attempts were made by MacKenna and his associates (*loc. cit.*) to determine the quantities of fat-soluble vitamins contained in sebum. They were unable to detect any vitamin A, beta-carotene, vitamin K, or provitamins D_2 and D_3 in the lipid extracts by the chemical procedures utilized. Small quantities of substances which reduce ferric chloride were found. The evidence indicated that some portion of these materials was very likely vitamin E, but that the quantity of tocopherol contained did not exceed 0.2 mg. per gram of sebum. Spectrophotometric assay was undertaken in another laboratory (G. N. Festenstein and R. A. Morton, *Biochem. J.* **52,** *168 (1952)*). The results of these determinations confirmed the absence of vitamin A, beta-carotene, vitamin K and provitamins D in the specimens of sebum examined. Conclusive evidence of the presence of vitamin E was not afforded, but if present it was estimated that the quantity did not exceed 0.3 mg. per gram of sebum.

The conclusion seems warranted that no significant quantity of the fat-soluble vitamins is lost to the body through the secretions of the sebaceous glands. Deserving special comment is the failure to demonstrate provitamins D_2 or D_3 in human sebum. Vitamin D has been shown to be formed from its precursors with irradiation of the skin of various animals. This had been presumed to be a process occurring at or very near the superficial layers of the skin. It would, therefore, be considered likely that sebum would contain provitamin D. Perhaps the failure of MacKenna and his associates (*loc. cit.*) to demonstrate these materials in sebum does not prove conclusively that they are not present in small quantities. As the authors themselves point out, one of the incompletely identified substances in the unsaponifiable fraction of sebum was a sterol-like substance. It is conceivable that this compound on further study may prove to be provitamin D.

DIET SODIUM AND ADRENAL HORMONE EFFECTS

The metabolic effects of adrenal cortical hormones have been popular subjects of study as each compound has become available for clinical investigation. It has been shown that cortisone, compound F and desoxycorticosterone acetate (DOCA) are capable of producing changes in carbohydrate, protein and electrolyte metabolism which differ not only in degree but, depending upon the hormone used, may be limited to only one portion of body metabolism (*Nutrition Reviews* **9,** *260 (1951)*). Thus DOCA has a potent sodium-retaining and potassium-diuretic effect, but produces no significant carbohydrate or protein alterations. With the increasing use of these agents in clinical medicine the undesirable metabolic sequelae have had to be seriously considered, but relatively little study has been devoted to the role of dietary constituents in the production or prevention of such sequelae.

Although DOCA has not been found particularly useful in treatment of any disease except adrenal insufficiency, a recent investigation of the role of sodium chloride intake in altering its effects in normal man may well serve as a pattern for such study of other adrenal hormones (A. S. Relman and W. B. Schwartz, *Yale J. Biol. Med.* **24,** *540 (1952)*). In this study sodium, potassium, chloride and nitrogen balances were carried out in three groups of normal subjects receiving 20 to 40 mg. of DOCA daily. One group of three subjects received a low sodium diet (13 to 15 mEq sodium per day), another received this diet plus 180 mEq of sodium (as sodium chloride), while the third group received the diet plus 40 mEq of sodium orally and 450 mEq intravenously as isotonic saline.

During the period of DOCA administration sodium retention and potassium loss (in excess of that accounted for by nitrogen loss) were noted in those subjects receiving sodium supplements, but were absent or very slight in the subjects given the diet alone. Although these changes were due primarily to altered renal excretion of electrolytes, similar changes were noted in fecal electrolytes. Nitrogen balance was unaffected, although there was a small but definite increase in urinary ammonia excretion during DOCA administration in all the subjects who were given salt. This was noted transiently and to a less marked degree in only one subject in the low sodium group. The sodium retention tended to diminish with continued DOCA administration. Ammonia excretion continued, however, and potassium excretion showed no reversion toward control levels except in 2 patients in whom this took place after seven and nine days of DOCA administration respectively. Reversal of all the changes noted occurred in all 5 subjects in whom the investigation was continued after the hormone was stopped.

Serum potassium levels were unchanged in the low-salt subjects, but fell significantly in all who were given salt, while sodium, chloride and pH of the serum were unaltered. Following cessation of DOCA administration, potassium concentration in the serum temporarily rose above control levels. In regard to shifts occurring between intracellular and extracellular compartments, the data indicated that sodium entered the cells and that there was loss of potassium from the cells but without quantitative relationship between these two electrolytes. These findings were also reversed after the DOCA was discontinued.

Alteration of diet sodium and potassium has been reported to be similarly effective in preventing the "toxic" effects of adrenal hormone therapy (L. Greenman *et al.*, *J. Clin. Invest.* **30,** *644 (1951)*). These workers substituted low sodium milk formulas for whole milk and were thus able to prevent edema formation, and to maintain positive potassium and nitrogen balances in patients receiving cortisone or ACTH.

Although the mechanisms responsible for the apparently beneficial effects of reduced sodium intakes during the administration of DOCA, cortisone and ACTH have not been elucidated, these studies suggest that compensatory mechanisms are present in the body which prevent potassium loss when allowed to operate in the milieu of a sodium-poor intake. That these mechanisms operate at least to some extent even when sodium is not restricted is indicated by the fact that reversion toward control levels of sodium and potassium excretion occurred in some patients studied by Relman and Schwartz even during continuation of DOCA administration. Similar stabilization of sodium and potassium excretion has been observed during cortisone therapy by R. G. Sprague *et al.*, (*Arch. Int. Med.* **85,** *199 (1950)*). It would appear that a possible mechanism of such compensatory changes is the secretion of other adrenal hormones which induce sodium excretion rather than retention (*Nutrition Reviews* **10,** *287 (1952)*).

VITAMIN B_{12} AND GROWTH IN CHILDREN

A great deal of interest in the role of vitamin B_{12} in producing acceleration in the growth of school children has followed the preliminary report of N. C. Wetzel, W. C. Fargo, I. H. Smith, and J. Helikson (*Science* **110,** *651 (1949)*; *Nutrition Reviews* **8,** *139 (1950)*). The niceties of the "Wetzel Grid Technique" make it exceptionally suitable for detecting changes in growth rates of school children over relatively short periods of time when the measurements of these children are of suitable accuracy. Further studies of growth failure in school children and the effect of dietary supplements of vitamin B_{12} have been reported by Wetzel, H. H. Hopwood, M. E. Kuechle, and R. N. Grueninger (*J. Clin. Nutrition* **1,** *17 (1952)*). In this review emphasis is placed upon the definition and occurrence of simple growth failure in school children.

The statement is made that ". . . simple growth failure even among children of high-income areas had been, and continues to be, on the increase," but the evidence for this conclusion is not presented. Simple growth failure is defined as a generalized deficiency in which physical growth is measurably below par. It is said to involve "both substance as well as fuel debts which must be made up before recovery is achieved" and is "an entity that is remarkably free from specific organ lesions or symptoms. Its outward signs are few: loss of physique, fatigue, and dry skin are but rarely so pronounced as to be recognized on their own." It is concluded that the use of the Grid is about the only way to illustrate clearly either the onset of or recovery from simple growth failure.

The present study was done in the Medical and Health Department of the Shaker Heights Schools in Cleveland which are located in a high-income suburban community not characterized by marginal living. Simple growth failure is said to be present in about one third of the children and honor students and champion athletes are not found in this group. The report implies that about one third of the children in similar areas may be expected to exhibit growth failure as measured by the Grid technic.

Essentially three groups were used. Twenty children not showing growth failure served as controls and two other groups of 20 and 16 were observed before and after treatment with 10 micrograms of vitamin B_{12} orally each day. For purposes of statistical analysis, the growth rates are defined in terms of developmental levels per month according to the Grid. Each individual serves as his own control and other members of the group serve as time, place, season, etc., controls.

In the 36 children who received vitamin

B_{12}, 23 showed an acceleration of growth after the vitamin B_{12} supplements were given. This average increase in growth, comparing rates before and after supplementation, was significant at the 0.1 per cent probability level in the group which received the supplement for sixteen weeks and at the 5.0 per cent level in the group which was given the supplements for only six weeks. Brief physical examinations showed no specific lesions that could be associated with a possible vitamin B_{12} deficiency.

Another study involving 236 children is briefly mentioned. Data are not presented, but it is stated that a highly significant growth response was found even when the whole group was used in the analysis. That is, when all of the responses, whether positive, zero, or negative, are averaged, there was a sufficient number of positive responses to give an increased growth rate for the group as a whole of 0.244 ± 0.067 levels per month. Whether this was a random sampling from the school, or children with growth failure only is not specified.

The authors conclude that "unless some extraordinary chance event, with a probability of less than 1 in 1000 trials had befallen this study, it is impossible to avoid the conclusion that the vitamin B_{12} supplements and the growth responses are related as cause and effect."

It will be agreed that there was a difference in growth rates before and after vitamin B_{12} supplements, but the report does not present convincing evidence that the growth change can be attributed to an effect of vitamin B_{12}. The description of methods and procedures does not allow the reader to feel confident of a critically designed test. For example, the data on the selection, numbers, and characterization of the groups receiving placebos and the ages of the children in the whole study are not given. The 4 unsupplemented children were "withdrawals" from one of the experimental groups for reasons not specified and the presence of bias might be expected.

The brief and uninformative description of the use of lactose placebos coupled with the evidences cited of great interest and cooperation in the study leave one with misgivings as to the full appreciation of the importance of the control of psychologic factors in the prosecution of the study. For example, E. M. Widdowson (*Lancet* **I**, *1316 (1951)*), in a report entitled "Mental Contentment and Physical Growth," demonstrates that the food habits and consequent growth of children are responsive to the attitudes of teachers and that these attitudes may obscure or negate nutrition experiments. From the data presented by Wetzel *et al.*, it is impossible to evaluate the part played by parent and teacher interest in contributing to the results of vitamin B_{12} supplementation.

Food records were kept on 45 of the children receiving vitamin B_{12} and showed a "distinct advantage" from the "standpoint of caloric intake, food choice, and diet composition" with an increased caloric intake of 246 ± 89 calories per day associated with the increased rate of growth. These observations would be more convincing if the methods of obtaining and computing the dietary intakes were given and if a control group receiving placebos without the knowledge of teachers, parent, or nutrition worker had been included in the dietary studies.

The statement is made that "the most immediate reactions were noted . . . by the personnel of the medical and nursing departments, but almost simultaneously by teachers in the classrooms who became aware that at least some of the pupils were improving in behavior, attitude, and scholastic work—or that they were showing less strain and fatigue, or, on the other hand, greater interest and attention, and hence better all-around progress than before." Unfortunately, this enthusiastic and encouraging statement is not supported by objective

data, and actually suggests faulty design of the experiment. The parents and teachers, even to attempt an unbiased appraisal, should not have known which children received vitamin B_{12} as compared with an approximately equal group receiving placebos.

The statements of these authors that a characteristic of simple growth failure in children is a tendency to become progressively worse, and that growth rates in terms of developmental levels per month are independent of such factors as sex, body measurements, and—up to maturity—age are not substantiated in the present paper. Consequently, the growth acceleration occurring at 10 ½ years of age, *i.e.*, about the time when growth normally begins to accelerate, in the specimen record on one of the girls in the study cannot be evaluated in terms of the more commonly used standards of growth. Indeed, until well presented, comparative analyses of growth of randomized samples of children of all ages are made by more orthodox methods against the Grid, the validity of the Grid standard cannot be accepted without some reservation. According to Wetzel's statement in the present paper only 2 out of 3 children follow the Grid pattern. It is difficult to believe that a standard which is met by but two thirds of the economically privileged children is valid. The serious importance of conclusions as to validity of this method places on the investigator the responsibility for providing a basis for a clearly objective appraisal of his methodology.

In this connection, A. Yankauer (*Pediatrics* **10**, *627 (1952)*), in discussing the use of the Grid to differentiate between the healthy and the unhealthy in groups of school children, states that "since there have been no objective studies of the over-all incidence of major health problems among a group of school children as related to the normality or abnormality of their grid patterns, this claim can neither be proved nor disproved."

Finally, regardless of the possible effects of vitamin B_{12} or any other nutritional supplement on accelerating growth in children, the old and important question may be raised as to whether slow growth in relation to any standard is always "growth failure" for the individual. Growth failure by definition is pathologic and deserves medical attention. That it occurs and is an important problem in the care of children is conceded. That the grid may assist in its recognition is also granted, but there is no reason to believe that it is infallible. This study fails to convince the reviewers that the one third of the population studied who failed to keep pace on the grid, actually did so because of growth failure. A response to vitamin B_{12} would suggest this, but would not prove it.

It may even be that children who normally grow slowly at certain age periods have health advantages. This is suggested by the well-known studies of C. M. McCay *et al.* (*see Nutrition Reviews* **2**, *3 (1944)*) which showed that in animals a slower rate of growth with some degree of caloric restriction promotes longevity. The advantages of rapid growth in hogs or broilers is obvious, but children deserve other considerations. On the other hand, many studies, especially those reported by H. C. Sherman after years of careful research, have failed to indicate any hazard to health and longevity as a result of rapid growth, except when the rapid growth induced a deficiency in another nutrient (*e.g.*, calcium).

The occurrence of periods of slow rates of growth in children in a well-to-do community is of interest; the long-range significance of the condition is as yet unclear and its nonspecific nature seems apparent. The role played by psychologic factors remains to be studied. Until more critical studies of the effect of vitamin B_{12} on the growth and learning of school children in growth failure are reported in the literature, the widespread use of this vitamin as a growth-promoting substance is not justified and should be discouraged.

DETERIORATION OF PERSONALITY IN EXPERIMENTAL STARVATION

The early publications on behavior in experimental semistarvation, studied toward the end of the Second World War at the University of Minnesota, have been reviewed (*Nutrition Reviews* **7,** *78 (1949)*). A systematic account of the psychologic aspects of the experiment was given in Chapters 36 to 42 of the collaborative treatise, "The Biology of Human Starvation" (A. Keys, *et al., Univ. of Minnesota Press, Minneapolis (1950)*).

The subjects were free from physiologic stresses other than those imposed by the dietary restrictions. In volunteering for the experiment they were strongly motivated by the desire to serve in a study designed to provide basic information about nutritional rehabilitation after starvation. As conscientious objectors they desired to match, in a way, the contribution of their contemporaries in military service.

However, psychologic stresses were not absent. They were separated from home and normal work or study environment. There were the emotional problems to be met by a conscientious objector at a time of war and, most importantly, the natural conflict between the need to exercise strict self-discipline in adhering to the severely restricted diet in the midst of plenty and the tendency to escape the rigors of the diet and its attendant discomfort.

While the accounts of overt behavior and personal experiences during natural starvation are abundant, records of starved explorers and victims of famines as well as the notes of relief workers are not systematic and do not provide basis for a quantitative treatment. Such records are notably weak as far as the rehabilitation phase is concerned; at times there was no rehabilitation to write about. The physiologic and, even more so, the psychologic effects of natural starvation are complicated by the superimposed hardships of non-nutritional character. Scientifically, perhaps the most severe limitation of field research consists in the fact that standardized psychometric tools and technics are hardly applicable under conditions of "real" starvation. Yet it is the introduction of systematic, quantitative methods of observation which makes the psychologic observations particularly useful in the context of nutritional research.

The technics used for the study of the effects of semistarvation on behavior and personality in the Minnesota study ranged from standardized tests and inventories through ratings by the experimenters and self-ratings of the subjects to the notes kept during interviews and the subjects' diaries. There was a marked elevation of the mean scores in the three "psychoneurotic" scales (hypochondriasis, depression and hysteria), constituting a psychometric index of "semistarvation neurosis." In order to determine which items contributed to the elevation of these scores, an analysis of the responses on the three scales was made (J. Brozek and N. K. Erickson, *J. Consult. Psychol.* **12,** *403 (1948)*). The "somatic" items referred to such symptoms as fatigability, weakness, lack of energy, decreased ability to work, dizzy spells, cold hands and feet, and paresthesias. The "psychic" items indicated lack of spontaneity, the subjects' impression of decreased intellective ability and poor judgment, inability to concentrate, and lack of interest in matters of sex, play, and recreation.

The investigators raised the question as to whether the scores, obtained from individuals whose physical status was profoundly altered, could be assigned the meaning attached to them in the ordinary psychiatric context in which one deals with physically normal people. A detailed item analysis revealed that the items contributing to the elevation of the psychoneurotic scores were noted by individuals with frank psychoneuroses, not complicated by physical abnormality, with frequency similar to that

found in the responses of the semistarvation subjects. Thus there were no fundamental differences between the answers given by individuals exposed to a prolonged, severe nutritional stress and patients with a clinical diagnosis of psychoneurosis.

A quantitative study of perception and association in the Minnesota semistarvation experiment was reported by J. Brozek, H. Guetzkow, M. V. Baldwin, and R. Cranston (*J. Personality* **19,** *246 (1951)*). Direct clinical observations indicated that preoccupation with thoughts of food was one of the striking changes accompanying prolonged food restriction, together with depression, narrowing of interests, social introversion, decrease in spontaneous activity —physical and mental—and a marked diminution of libido. The subjects talked and read about food. In the movies, their attention was attracted by the scenes of food and eating. Cook books, menus, even such dry matter as information bulletins on food production became fascinating subjects to men who, a few months earlier, gave little thought to the fine points of culinary art or to the intricacies of agricultural statistics. Strangely enough, the increased hunger drive and the heightened emotional significance of food was not reflected either in the perceptual tests or in a systematic analysis of the content of the dreams. Only one of the association tests provided limited evidence of semistarvation alterations. The effects were fairly specific. Semistarvation influenced chiefly reactions to those stimulus words whose content had to do with eating (such as bitter, thirsty, and cabbage).

It is somewhat surprising that the fraction of responses related to food was identical in the experimental and in a well-fed control group (5.9 and 5.8 per cent). In view of the intense preoccupation of the semistarvation subjects with food one might expect a greater number of food-related responses. The data did not support such a hypothesis. Also surprising was the absence of an increase in the frequency of food dreams recorded at intervals throughout starvation and the early phase of nutritional rehabilitation. Reference to "frequent" food dreams are found in the reports of the victims of famine and other forms of natural starvation, and if dreams are to be interpreted, with the psychoanalysts, as a release of drives and strivings which cannot find fulfillment in real life, one would expect the starving man to dream about food and eating more than when well fed. An analysis of a random, unbiased sample of dreams did not substantiate the expectation. However, food dreams were mentioned in the subjects' diaries and a good deal of daydreaming was devoted to matters of food.

In this context A. R. Holmberg's observations on the Siriono tribe of Eastern Bolivia ("Nomads of the Long Bow," *Smithsonian Institution Inst. Soc. Anthropol. Publ. No. 10 (1950)*) are of interest. The author's central hypothesis was that under conditions of sparse and insecure food supply the anxieties and frustrations related to the "hunger drive," the strongest biologic motivating force, should find repercussions in behavior, personality, and culture. Of some fifty dreams, recorded by the author, more than one-half were related to eating, the hunting of game, and the collecting of edible products from the forest (*loc. cit., p. 91*). This would seem to fit perfectly the hypothesis. However, it was noted specifically that the food dreams seemed to occur just about as often when a person was not hungry as when the individual was hungry. Thus the food content of the dreams appears to have a cultural rather than physiologic etiology.

The results of two standardized projective tests applied in the Minnesota starvation-rehabilitation experiment, Rosenzweig's picture-frustration test (J. C. Franklin and J. Brozek, *J. Consult. Psychol.* **13,** *293 (1949)*) and the Rorschach test (N. K. Kjenaas and Brozek, *Psychosomatic Med.* **14,** *115 (1952)*), were essentially negative. In the former test, which consists of roughly-drawn

cartoons which illustrate common frustrating situations and requires the subject to supply the omitted dialogue, there was no significant change in the quality of the responses after twelve weeks of nutritional rehabilitation and the four caloric levels of refeeding were not reflected in either response criterion (type of reaction and direction of aggression). In view of the unquestionable frustration of the hunger (food) drive in starvation and its reduction on refeeding, the data raise fundamental questions concerning the validity of the concept and method of personality appraisal by this (and other) "projective" technics. In this way the "applied" nutritional research serves as stimulus for personality theory.

The Rorschach test was administered to a group at the control period, after twenty-four weeks of semistarvation and twelve weeks of rehabilitation. In starvation there was an increase in the majority of the response categories, but the increases were proportional to the over-all increase in the number of responses (interpretations of ink blots) which was considered to represent a retest phenomenon. There was no significant difference in the starvation and rehabilitation group "profile" in which the frequency of the individual Rorschach variables (such as large detail, human movement, food) was expressed as percentage of the total number of responses. Of theoretic interest is the attempt to predict resistance to deterioration under the stress of semistarvation, based on Rorschach responses made during the control period.

These studies, taken as a whole, indicate at the same time the usefulness of psychologic measurements in nutritional investigations on man and the limitations of available methods for personality appraisal.

THE USE OF INTRAVENOUS IRON

Iron in various forms and combinations has a prominent place in medicine. The use of oral preparations of iron in the treatment of iron deficiency anemia is well established. Parenteral use of this element in treatment of hypochromic anemia has been limited by the incidence of toxic reactions. The intravenous use of saccharated iron oxide in the treatment of hypochromic anemia was introduced in 1947. This compound is a negatively charged colloidal solution of iron oxyhydrate stabilized by adsorption of alkali and sugar.

In a recent article J. E. Lucas and A. B. Hagedorn (*Blood* **7**, *358* (*1952*)) discuss the use of high molecular ferric carbohydrate in treating 20 patients suffering from various hypochromic anemias. Further evaluation in 45 patients is presented by Hagedorn (*Proc. Staff Meetings Mayo Clinic* **27**, *277* (*1952*)). Both studies showed similar results.

Patients were selected who met the following criteria: (1) hemoglobin 10.5 g. per 100 ml. of blood or less, (2) hypochromasia in the peripheral blood smear, and (3) mean corpuscular hemoglobin concentration of 30 per cent or less.

The total of elemental iron required in each case was approximated by assuming that 25 mg. were required to raise the value of hemoglobin 0.15 g. per 100 ml. of blood. Doses of iron were usually 50 mg. initially, then 100 mg. once or twice daily thereafter. Larger doses produced more reactions. No fatal reactions at any dose level have been reported.

The average rate of maximal regeneration of hemoglobin was 0.26 g. per 100 ml. per twenty-four hours in patients with initial values of less than 8 g. In patients with 8 g. of hemoglobin or more this increase was 0.16 g. Reticulocytes reached a peak in five to seventeen days after initiation of treatment. Hemoglobin regeneration is more rapid than in comparable cases receiving oral preparations of iron.

Elemental iron given intravenously is almost quantitatively utilized in the formation of new hemoglobin in anemia caused by a deficiency of iron. The immediate hematologic effects of intravenous saccharated iron oxide were studied by L. P. White, H. R. Bierman, K. H. Kelly, and R. L. Byron (*Blood* **7,** *897 (1952)*). They investigated the mechanism of the production of a sudden leukopenia that occurred following the intravenous injection of saccharated iron oxide in colloidal suspension.

This leukopenia involved all types of white cells and occurred promptly in the arterial blood followed by a similar fall in the venous blood within ninety seconds. The magnitude of the fall in leucocytes was directly proportional to the amount of material injected, 100 to 1000 mg., and was independent of the rate of injection.

The authors hypothesize that the leucocytes are temporarily stored in the pulmonary vascular system. Following the cessation of the leukopenic stimulus probably many of these same cells are returned to the general circulation from the lungs within minutes. The higher leucocyte counts in the arterial blood during recovery from leukopenia support this hypothesis. Since other colloidal substances have been found to produce the same phenomenon, the colloidal nature of iron may be involved in producing the leukopenia. The rôle of vasodilatation in this leukopenia is uncertain since all patients receiving large amounts of iron showed evidence of vasodilatation.

There was no significant change in the platelet number in arterial and venous blood. An increased clotting power of venous blood containing large amounts of iron was observed in 2 patients only during the period of injection.

Two toxic reactions were encountered: (1) local inflammation when the iron preparation was injected paravenously; and (2) flushing of the face, nausea, headache and abdominal cramps when the injection was too rapid. Aching and stiffness in the back and extremities, nausea, and abdominal cramps were noted as a delayed reaction. The greater incidence of toxic reactions after the intravenous use of iron as compared to the incidence after oral administration favor the use of oral preparations in a given situation.

Iron which is given intravenously is retained almost quantitatively by the body. Large amounts given to animals have caused no structural changes in the tissues; however, excessive deposits of iron during the years may produce damage to the tissue. Care is necessary in the intravenous administration of iron.

According to Hagedorn, the indications for the intravenous use of iron are: (1) uncomplicated anemia caused by deficiency in iron that has been unresponsive to prolonged periods of adequate oral administration; (2) intolerance to iron given orally; (3) allergy or sensitivity to oral iron (a rare situation) when desensitivity may be accomplished by iron given intravenously; (4) anemia caused by deficiency in iron discovered too late in pregnancy for administration of adequate amounts of iron by mouth, and (5) severe hemorrhage in patients in whom transfusions are prohibited for religious reasons.

TRACE ELEMENTS AND BOVINE BRUCELLOSIS

Trace elements, such as copper and cobalt, are known to be highly important in the nutrition of cattle. Over the past fifteen or twenty years, or since the discovery of the importance of the trace elements in bovine nutrition, trace mineral mixes have been sold to farmers. Usually the prices on such products have been high and many claims as to their value in various disease conditions have proven unfounded. What

appears to be a case in point is the relatively recent claims of "Bang's disease cures," which have resulted in the sale of considerable quantities of trace element mixtures. In this particular instance the claim was advanced that manganese would be of value in the treatment of Bang's disease or brucellosis because the spectroscopic analysis of the blood and pituitary glands of cattle reacting to the agglutination test for brucellosis revealed reduced concentrations of manganese, copper and cobalt.

Workers at the University of Wisconsin have recently conducted studies to test the influence of trace elements in the ration on the susceptibility of cattle to infection with *Brucella abortus* and to determine the therapeutic effect of trace elements in cattle infected with this organism (D. T. Berman, P. H. Phillips, and C. A. Brandly, *J. Am. Vet. Med. Assn.* **121,** *46 (1952)*).

Forty-one dairy heifers, free from *Brucella* infection, were fed a practical dairy ration which consisted of mixed legume hay, corn silage, and mixed grains. Nineteen of the animals received a supplement of trace elements mixed with the grains to supply approximately 1 ounce daily during nonpregnancy, and approximately 1.7 ounces during pregnancy. The trace elements supplement was prepared as follows (in pounds): 65 per cent manganese sulphate (22 per cent manganese), 100.0; copper sulfate, 1.0; cobalt sulfate, 1.5; zinc sulfate, 0.5; and magnesium sulfate, 3.0. All animals were exposed to *Brucella abortus* by instillation of a constant dose of the organism into the conjunctival sac four and one-half months after the start of the experiment. Most of the animals were three to six months pregnant at the time of exposure. The experiment was continued for a seventeen-month period after they were exposed.

The abortion rates and the infection rates were similar in the trace element-supplemented and the control group. Neither were any differences observed between the two groups in the agglutination reactions during the postexposure period.

The results of this study demonstrate that the supplementation of a practical dairy ration with trace minerals, in proportions recommended by those who have made claims for benefits from such supplements, had neither a prophylactic nor a therapeutic effect on the cause of experimentally induced brucellosis in cattle.

The authors cite a personal communication from G. K. Davis of the Florida Agricultural Experiment Station who was likewise unable to demonstrate any influence of manganese supplementation in bovine brucellosis. Davis' study was conducted on 27 cattle herds located in the manganese-deficient areas of Florida.

KETOSIS IN DAIRY CATTLE

Many investigators have studied ketosis, or acetonemia, in dairy cattle over the past twenty-five years. The common symptoms of this condition in cattle are rapid loss of body weight, anorexia and unsteady gait. Biochemical tests on cattle with ketosis reveal hypoglycemia, a relatively high glucose tolerance and the presence of increased amounts of ketone bodies in the urine. As a rule, this condition is observed in cows only during pregnancy or lactation, and this suggests that it is a metabolic disturbance brought about by the physiologic stress associated with the reproductive process. The urine test for ketone bodies is a reliable means of detecting ketosis in cattle before the condition progresses to the damaging stage (J. Sampson, *J. Am. Vet. Med. Assn.* **121,** *25 (1952)*).

One of the important recent contributions to this problem is the observation by J. C. Shaw, B. C. Hatziolos, and E. C. Leffil that

cortisone and ACTH are beneficial in the treatment of ketosis in dairy cattle (*J. Am. Vet. Med. Assn.* **117,** *103 (1950)*). It was postulated that this disease in dairy cattle is ordinarily caused by an adrenal insufficiency involving the adrenal cortex and the anterior lobe of the pituitary gland (Shaw, Hatziolos, and A. C. Chung, *Science* **114,** *574 (1951)*; G. Puntriano, *Vet. Med.* **46,** *215 (1951)*).

The latter investigator has recently reported a study in which data are presented supporting this postulate. Puntriano used a bioassay procedure with mice to measure the urinary output of adrenocorticoid hormone in normal cows and in cows suffering from ketosis (*Am. J. Vet. Res.* **13,** *129 (1952)*). Extracts of urine in 5 per cent glucose solution were injected into fasted, adrenalectomized mice, five injections per mouse, at one-hour intervals. The mice were killed one hour after the last injection, and the liver glycogen concentration was determined. Adrenocorticoid hormone under these conditions is known to stimulate the deposition of liver glycogen in fasted adrenalectomized animals. The procedure is thus an indirect measure of adrenal cortical hormone.

Two normal lactating cows possessed what was assumed to be normal urinary adrenocorticoid activity. Twenty-four-hour urine extracts increased the liver glycogen in the fasted adrenalectomized mice from the control level of 3 mg. per 100 g. of mouse body weight to 13.0 and 11.3 mg., respectively.

Two normal pregnant cows (pregnancy approximately 8 months) exhibited elevated adrenocorticoid activity. The liver glycogen in the test mice was increased to 21.3 and 18.9 mg. per 100 g. of mouse body weight, respectively, when injected with twenty-four-hour urine extracts from these animals.

Two cows suffering from ketosis possessed a greatly reduced urinary adrenocorticoid activity. Twenty-four-hour urine extracts from these animals resulted in average liver glycogen depositions of only 6.3 and 5.0 mg. per 100 g. body weight, respectively, in the test mice. This represented a significant reduction in the potency of adrenal cortical hormones in the urine, and supports the hypothesis that adrenal cortical insufficiency is indeed an important expression of ketosis in cattle.

The increased adrenocorticoid activity of the urine of pregnant cows was interpreted as an expression of intense physiologic stress.

As pointed out recently by Sampson (*loc. cit.*), the control of ketosis in dairy cattle becomes more difficult in the dairy industry because of the emphasis of the animal breeder and the animal nutritionist on increased milk production. Ketosis is particularly likely to occur in the animals of a herd that are the most prolific milk producers. The presently reported results by Puntriano suggest that the emphasis on high milk yield in dairy animals produces a physiologic stress that results in exhaustion of available adrenal cortical hormone. It is well known that a liberal energy intake is important in the prevention of ketosis in heavy milk production. The ideal nutritional regimen conducive to the maintenance of adequate adrenal function under such conditions deserves the attention of dairy nutritionists.

NUTRITIVE VALUE OF CORN

Considerable work has been reported on various factors affecting the nutritive value of corn since a recent review appeared on this subject (*Nutrition Reviews* **10,** *70 (1952)*). P. A. Miller and B. Brimhall (*Agronomy J.* **43,** *305 (1951)*) reported a study of factors influencing the oil and protein contents of corn grain. They found that

increased oil content in the grain depends primarily on increased proportion of germ in the kernel as well as increased concentration of oil in the germ itself. Variation of total oil percentage is not associated with total protein content, but is positively correlated with percentage protein in the germ and with the relative concentration of tryptophan in the entire kernel. From the genetic viewpoint it was interesting that the ear-bearing parent has the predominant influence on the oil content of the grain, while the source of pollen has but little effect. The oil content of corn grain may be increased as much as 3 per cent without loss in yield.

Additional studies of the relative effectiveness of two systems of selection for oil content were described by G. F. Sprague, P. A. Miller, and B. Brimhall (*Agronomy J.* **44,** *329* (*1952*)). The recurrent selection system and selection in selfed lines were compared for their relative efficiency in increasing the oil percentage of corn grain. At the end of the experiment, the recurrent series had passed through two complete cycles and the inbred series had been self-pollinated for 5 generations. The former selection system was more effective by factors ranging from 1.3 to 3.0, depending on the particular contrast used. It was pointed out that any of the contrasts studied minimize the efficiency of recurrent selection, since they disregard the differences in residual genetic variability. To conserve genetic variability, it was proposed that at least one generation of random mating after intercrossing might increase the efficiency of recurrent selection. Appreciable improvements in oil content seem practical after a sufficient understanding of the fundamentals of the heredity of this and associated characters has been gained.

A long series of 50 generations of selection at the Illinois Agricultural Experiment Station for high and low protein and high and low oil contents in corn was completed in 1949. Results are summarized by C. M. Woodworth, E. R. Leng, and R. W. Jugenheimer (*Agronomy J.* **44,** *60* (*1952*)). These studies began in 1896 on the variety Burr White. Ear-to-row selection was practiced for the first 28 generations; then mass selection with intrastrain controlled cross-pollination was employed for the last 22 generations.

The original variety had a mean oil content of 4.70 per cent and a mean protein content of 10.92 per cent. After 50 generations of selection, the mean oil content of Illinois High Oil was 15.36 per cent and that of Illinois Low Oil 1.01 per cent; the mean protein content of Illinois High Protein was 19.45 per cent and that of Illinois Low Protein was 4.91 per cent. These gains percentagewise in the high strains are by factors of about three and two for oil and protein, respectively. Progress is still being made in the High Oil and Low Protein strains, while little progress was made in either the High Protein or Low Oil strains in the last 15 to 20 generations of selection. Variability has remained unchanged in High Oil. High Protein and Low Oil have increased and Low Protein has decreased in variability.

During 8 generations from 1934 to 1941, a parallel random breeding experiment was conducted, in which chemical composition did not guide the choice of seed ears for each new generation. In all four strains, the nonselected groups showed some tendency to revert toward the composition of the original Burr White. This tendency was greatest in High Oil. In 1948, experiments in reverse selection were started. Two generations indicated that no change occurred in the oil content of Low Oil by reverse selection, but all other strains possessed considerable genetic variability.

All selected strains have been considerably modified in numerous morphologic and agronomic characters; each strain has a distinctive kernel type, ear type, cob type, maturity, plant height, ear height, tillering tendency, and susceptibility to leaf firing.

Grain yields of all four strains have been reduced to about 50 per cent of those of standard adapted hybrids. Despite this low yield, High Oil produces more pounds of oil per acre than commercial hybrids or the best three-way crosses involving one High Oil line. High protein was exceeded in total protein per acre by both standard hybrids and high protein three-way crosses. These high strains are being used by experiment stations to transfer high oil or protein to standard inbred lines.

P. A. Miller, T. L. Hurst, and B. Brimhall (*Agronomy J.* **44,** *343* (*1952*)) analyzed Illinois High Protein, Illinois Low Protein and the F_1, F_2, and backcross generations from the cross of these two for protein, zein, tryptophan, lysine, valine, leucine, isoleucine and niacin. They report that a good opportunity exists for selection of corn strains with a more favorable lysine-zein ratio. Selection for increased crude protein content may result primarily in an increase of zein, a very low quality protein. Perhaps it would be more desirable to select for increased percentage of lysine and tryptophan. Since addition of niacin to corn rations may increase the utilization of tryptophan by animals, breeding programs might wisely include selection for niacin. Content of niacin appeared in this study to be independent of protein and the protein components studied.

A study of the correlation between vitamin content and protein content of corn was made by E. B. Earley, J. N. Carter, and B. C. Johnson (*Agronomy J.* **44,** *326* (*1952*)). They studied concentrations of phosphorus, nicotinic acid, thiamine and carotene in kernels of a medium-protein and Illinois High Protein corn at several stages of development grown on two levels of soil nitrogen and phosphorus. The concentrations of all these substances, with the exception of carotene, were highest in the young kernels and lowest in the fully mature ones of both strains. Carotene was too low for the changes to be very significant from the practical standpoint. Heavy soil applications of nitrogen and phosphorus resulted in an increase in protein, phosphorus and thiamine and a decrease in nicotinic acid in mature kernels of both strains. The high protein corn was very superior to the other variety for both levels of soil fertility because its mature kernels contained 101 per cent more protein, 49 per cent more phosphorus, 45 per cent more nicotinic acid and 27 per cent more thiamine than the medium-protein corn. Some of these results differ from those of previous reports on other corn varieties.

NUTRITION STUDIES WITH THE NEW WORLD MONKEY

Nutrition research with primates, other than that conducted with human beings, has been limited almost entirely to studies with the Old World macaque monkey (genus *Macaca* and *Mulatta*). The use of the Old World monkey in nutrition research has had disadvantages. These animals must be imported from great distances and they are quite susceptible to tuberculosis and other diseases.

Nutritionists at the Harvard School of Public Health have turned to some studies with the New World monkey (Cebus monkey) to broaden our knowledge of primate nutrition (G. V. Mann, P. L. Watson, and L. Adams, *J. Nutrition* **47,** *213* (*1952*)). For the past three years these investigators have studied the nutritional requirements and the manifestations of specific nutritional deficiencies in Cebus monkeys.

The animals were procured from South America, from whence they are readily available and can be shipped by air freight. The Cebus monkeys weighed from 800 to 1500 g., as obtained in the partially grown state, and were found to be approximately

2000 g. in body weight when fully grown. They were found to be free of ectoparasites and of tuberculosis. A prevalent metazoic parasite found in nearly all the monkeys was the filaria, tentatively *Acanthocheilonema perstans*, and treatment with hetrazan was not successful.

The Harvard investigators noted that the Cebus monkey could be maintained in good nutritional health for periods of three years on 50 to 90 g. daily of a purified diet of the following percentage composition: vitamin-free casein, 25; sucrose, 60.6; corn oil, 8; cod liver oil, 2; salts, 4; para-aminobenzoic acid, 0.1; inositol, 0.1; and choline chloride, 0.2. The daily allowance of vitamins for each monkey consisted of the following (in milligrams): thiamine hydrochloride, 1.0; riboflavin, 1.0; pyridoxine hydrochloride, 1.0; niacin, 4.9; calcium pantothenate, 3.0; folacin, 0.1; biotin, 0.02; and ascorbic acid, 25.0.

Young animals placed on this diet when 1200 g. in body weight grew at a normal rate and increased their weight approximately 400 g. in a period of ten to fifteen months. It was pointed out that one of the disadvantages of use of these animals for nutritional experimentation is still, as in other monkeys, the small growth potential that remains when the animals are received. The animals as received were presumed to be from 1 to 2 years of age.

Physiologic measurements were made in order to obtain some information on the variability between animals. Blood plasma glucose was found to be 68.9 mg. per cent (standard deviation, 24.6). Other plasma values (with standard deviations given in parentheses) were as follows, in milligrams per cent: total cholesterol, 92.5 (31.5); free cholesterol, 24.6 (11.6); and nonprotein nitrogen, 34.1 (10.0). Total plasma protein averaged 7.66 (0.47) g. per 100 ml. of plasma, with 3.64 (0.47) g. as albumin and 3.50 (0.67) g. as globulin. Total plasma riboflavin was found to average 3.78 (1.17) micrograms per cent, the flavin adenine dinucleotide 2.65 (1.03), and the free riboflavin plus flavin mononucleotide 1.15 (0.36).

Blood hemoglobin values were found to average 16.58 g. per 100 ml. of blood and red blood cell counts averaged 6.98 million per cubic millimeter. It was noted that hematologic values were subnormal when the animals were received, and that usually about one month of feeding on the purified diet was necessary for stabilization at normal values.

In another paper (G. V. Mann, P. L. Watson, A. McNally, and J. Goddard, *J. Nutrition* **47,** *225 (1952)*) the Harvard workers reported studies on riboflavin deficiency in the Cebus monkey. The object of this study was to determine whether blood levels of riboflavin and its derivatives could serve as a reliable measure of riboflavin deficiency. There is a real need for reliability in diagnostic procedure since in clinical medicine the diagnosis of riboflavin deficiency is based upon such vague signs as cheilosis, magenta tongue and seborrheic dermatitis. Level of urinary excretion of riboflavin has been proposed as an aid in the diagnosis of ariboflavinosis in man (*Nutrition Reviews* **9,** *49 (1951)*) and results presented by H. B. Burch, O. A. Bessey, and O. H. Lowry (*J. Biol. Chem.* **175,** *457 (1948)*) suggested that riboflavin blood levels might be used in the diagnosis of ariboflavinosis in man.

Riboflavin deficiency in the Cebus monkey was characterized by a very rapid decline in the blood levels of riboflavin and its derivatives. This occurred within one or two weeks after instituting the feeding of the purified diet without riboflavin supplement. The flavin adenine dinucleotide fraction appeared to be the most reliable as a measure of ariboflavinosis and this component was the last to return to normal levels when the diet was supplemented with riboflavin. The normal value for flavin adenine dinucleotide, 3.24 micrograms per 100 ml. of plasma, usually declined to about 1.0 microgram on the deficient diet and remained at that level. Since there was little or no change

in the plasma flavin fractions after the initial sharp decline, this test did not allow predictions of the final failure that led to death. It did, however, serve as a reliable indicator of the onset or presence of riboflavin deficiency. The free plasma flavin was a less sensitive indicator than the dinucleotide fraction because of its greater variability.

The riboflavin requirement for the Cebus monkey appeared to be between 25 and 35 micrograms per kilogram of body weight, which compares favorably with values reported for other mammalian species.

The studies with the Cebus monkey emphasize again the inconsistencies of dermal and other grossly visible lesions as manifestations of riboflavin deficiency. Nonspecific changes observed were loss of weight, scaly dermatitis, and loss of hair. Neither oral nor ocular lesions were observed, and anemia was not present, even in the terminal stages. The fatty "yellow" livers that have been reported to occur in riboflavin deficiency in dogs and macaque monkeys (J. M. Cooperman, H. A. Waisman, K. B. McCall, and C. A. Elvehjem, *J. Nutrition* **30,** *45 (1945)*) were likewise not seen in the riboflavin-deficient Cebus monkeys.

These studies suggest that the Cebus monkey will be a useful animal for extending our knowledge of the nutrition of primates.

THE ORGANIC MATRIX OF ENAMEL

Reference has been made previously to the observation that the hardest, most highly calcified structure of the body, the enamel of the teeth, contains a finely dispersed honeycomb-like organic matrix (*Nutrition Reviews* **6,** *70 (1948)*). This is contrary to numerous earlier opinions that it was entirely inorganic, or that whatever traces of organic matter which might be contained therein had no specific organization. At the level of resolution and inspection permitted by the light microscope, this organic matrix seemed to have as its finest subdivision the discrete prism sheath which surrounded each of the millions of enamel prisms which are the major building blocks in any enamel crown. Furthermore, it has been shown that characteristic staining reactions can be demonstrated in this organic matrix which enable the investigator to describe in approximate fashion the type of organic compounds present therein (*Ibid.* **9,** *268 (1951)*).

The development of replica technics of and ultrathin sectioning procedures which could be applied to teeth made possible electron microscopic studies of the distribution of the organic and inorganic elements within the enamel (D. B. Scott, M. J. Ussing, R. F. Sognnaes, and R. W. G. Wyckoff, *J. Dent. Res.* **31,** *74 (1952)*). The electron micrographs of these preparations demonstrated that, in addition to the previously described honeycomb-like organic structure, there was an extremely fine submicroscopic fibrillar network which permeated the enamel prism and appeared as a filamentous spongelike network. Indeed, instead of the prism sheaths being a solid sheet as had been previously suspected, they actually were composed of a slightly more dense, closely packed fibrillar network than the one within the prisms. These fibrillar networks showed up particularly well in the decalcified sections where all or the majority of the salts had been removed prior to sectioning. The previous inability to visualize these networks was readily explained by the fact that the fibrils themselves were only a few hundred Ångströms in diameter; *i.e.*, well below the resolving power of the light microscope.

The inorganic crystals in fully mature enamel could not be visualized by the electron micrographs. The authors postulated that the dimensions of the network might indicate the maximum size attained by

crystals of the inorganic component, but it could not be definitely established that these elements were single crystals and not crystalline aggregates. The openings in the network averaged 200 millimicrons in diameter, with a range from 150 to 300 millimicrons. These values compared favorably with the mean size of 270 millimicrons calculated from x-ray diffraction studies (W. F. Bale, H. C. Hodge, and S. L. Warren, *Am. J. Roentgenol.* **32,** *369 (1934)*).

The enamel of mouse and monkey teeth has also been studied at various stages in development (R. F. Sognnaes, D. B. Scott, M. J. Ussing, and R. W. G. Wyckoff, *J. Dent. Res.* **31,** *85 (1952)*). From these studies it was concluded that the submicroscopic fibrillar organic network which permeates the mature enamel was not present as such when the enamel matrix of the developing tooth was laid down. The young enamel matrix was almost completely homogeneous. Slightly later in development there was a period in which the beginnings of the fibrillar network were evident. Progressively with development, the extent of the fibrillar nature of the mature enamel became more evident until at maturity the widely spaced, thinner network was achieved. The organic matter in the interprismatic sheaths remained homogeneous until a much later stage of development than the matrix within the prisms. Eventually the fibrillar network became evident in the interprismatic sheaths, and by the time the enamel was mature they had become approximately as fibrillar in nature as the organic substance within the prisms. It was stated that it was impossible to tell from these studies whether the alterations in the organic matrix were caused directly by the ingress and expanding growth of the crystalline materials, or whether there were chemical reorientations which provide space for the growing crystals along with a condensation and increase in density of the organic matter.

A great deal of further information needs to be sought about this organic framework. From histochemical staining reactions we know that it probably contains a substantial amount of keratin or some closely related keratin-like protein, an acid mucopolysaccharide, and probably alkaline phosphatase and traces of lipids. Yet this is far from a full knowledge of the composition of its components and the individual importance of each of the components. Furthermore, what is the status of these organic moieties with respect to their turnover rate? In view of their location in a heavily calcified structure, are they completely static or is there a potentiality for their replacement, as seems to be the case for organic components of other parts of the body? In addition, are these fibrils permeable to the passage of ions or dissolved molecules in the body fluids?

THE REGULATION OF FOOD INTAKE AFTER HYPOTHALAMIC LESIONS

The regulation of food intake by the animal organism is a fundamental physiologic process which is poorly understood. Ideally a delicate balance is maintained between caloric intake and caloric requirements for growth and repair. Disorders of this regulatory mechanism can lead to excessive leanness or obesity. A dramatic pathologic increase in food intake can be produced experimentally in a number of mammalian species by hypothalamic lesions (*Nutrition Reviews* **2,** *105 (1944)*). The most widely held explanation of this phenomenon has been that the operation blocks inhibitory influences from acting on hypothalamic appetite centers. The observed voracity of such experimental animals represents a release of these inhibitions. The metabolic consequences of the operation have been interpreted as the result of greatly increased

food intake (J. R. Brobeck, J. Tepperman, and C. N. H. Long, *Yale J. Biol. Med.* **15,** *893 (1942/43)*).

J. Mayer and M. W. Bates (*Am. J. Physiol.* **168,** *812 (1952)*; *Nutrition Reviews* **10,** *280 (1952)*) have suggested that food intakes are determined by the response of cerebral centers to the level of blood glucose. They were led to this conclusion by experiments on rats in which they measured the food intake of animals receiving injections of glucose, fructose, sucrose, fat emulsion and adrenalin. Those substances which are known to elevate blood glucose, namely glucose, fructose and adrenalin, caused a significant lowering of food intake even when the caloric equivalent of the injected sugar was considered. Sucrose and fat emulsion produced no significant change in food intake. Moreover, Mayer and Bates were able to show that injection of insulin producing a slight lowering of blood sugar, within the normal range and without hypoglycemia, was an effective agent in increasing food intake.

From these and other observations the conclusion was reached that alterations of blood sugar within the generally accepted normal range might still be an effective stimulus for increasing or decreasing food intake. In formulating a "glucostatic" theory the authors postulated that the regulation of food intake is determined by the necessity of maintaining a constant level of "effective" blood sugar. Not only the absolute level of blood sugar but also disorders of glucose utilization involving the "glucoreceptors" determines "effective" blood sugar.

The behavior of hyperphagic rats with hypothalamic lesions has been studied in respect to the glucostatic theory of food intake by J. Mayer, M. W. Bates, and T. B. Van Itallie (*Metabolism* **1,** *340 (1952)*). The operative procedure was performed with the Krieg stereotaxic instrument which is a modification of the Horsley-Clarke apparatus. Three to four weeks after the operation a careful correlation of blood sugar and weight gain was made. The authors emphasize that their observations were made during a period of stabilized hyperphagia and should not be compared with previous reports concerning the carbohydrate metabolism immediately after operation or during the late static phase of long-established obesity.

Blood sugar determinations were made at the same time of day in hyperphagic rats (food intake more than 50 per cent greater than preoperative intake), in rats operated upon but without hyperphagia, and in normal control rats, all of which had free access to food. The mean blood sugar for the three groups (standard deviations in parentheses) was respectively 92.2 (7.4) mg. per cent, 101.9 (7.0) mg. per cent and 102.7 (10.1) mg. per cent. Analysis of these data showed the difference between the hyperphagic group and the other two groups to be significant.

The data were also treated in two additional ways. When the blood glucose was plotted against average weight gain for the whole postoperative period of approximately one month a correlation was obtained (−0.454) showing that the greatest gains in weight occurred with the lowest blood sugars. A similar plot of blood sugar against the weight change for the four-day period, when the glucose sample was drawn, revealed an even closer correlation (−0.722) in the same direction. Considerable overlapping of the blood sugar of rats in the normal, nonhyperphagic operated rats and hyperphagic operated rats existed, but not a single actively hyperphagic animal had a blood sugar level as high as the average normal control level.

In an attempt to explain the above findings the insulin sensitivity of the experimental animals was tested, but the results were inconstant. Possible inactivity of the adrenal cortex was considered. Corticotrophin abolished the excessive weight gain and resulted in an increase in blood sugar

in the hyperphagic rats from 93 to 111 mg. per cent. The eosinophil count of the hyperphagic rats was higher than that of the control rats.

The small absolute decrease of the blood glucose in the rats with hypothalamic lesions assumes greater significance when compared to the blood sugar of force-fed normal rats. For example, F. L. Engel, A. Viau, W. Coggins, and W. S. Lynn (*Endocrinology* **50,** *100 (1952)*) found an elevation in blood sugar in rats which had been force-fed a high carbohydrate diet.

The essential and still largely unsolved problem presented by the results of Mayer *et al.* is to decide whether the observed changes in carbohydrate metabolism are the cause rather than the result of the hyperphagia. The authors have ably mobilized the arguments supporting their contention that the lowered blood sugar promoted the increased food intake. The immediate postoperative voracity, they admit, could be produced by the temporary release of the hypothalamus from inhibitory centers and might be independent of blood sugar. Several possible hormonal and nervous mechanisms were suggested whereby hypothalamic lesions could lower blood glucose level.

The glucostatic theory of the regulation of food intake has provided a provocative explanation for the hyperphagia following hypothalamic lesions. To gain widespread acceptance of this view additional experimental evidence must be obtained elucidating the mechanism whereby the blood sugar of these operated animals remains low despite increased food intake.

ADAPTATION OF TISSUE ENZYME ACTIVITY TO CHANGES IN DIET

Whether there exists a direct relationship between the type and quantity of certain tissue enzymes on the one hand and the quantity and quality of the food ingested on the other has long been a subject of controversy. The experimental observations which pertain to this relationship particularly as it relates to dietary protein and activity of a variety of enzymes in liver were reviewed recently (*Nutrition Reviews* **9,** *103 (1951)*). The evidence then available indicated that there is an important relationship between the quantity of protein in the diet and the activity of a number of enzyme systems in the liver, but that liver-enzyme proteins enter into large body metabolic pools of protein in a dynamic but non-specific manner.

In an effort to describe and characterize further this phenomenon of biochemical or enzyme adaptation, J. Mandelstam (*Biochem. J.* **51,** *674 (1952)*) has proposed a theory and model of adaptation by "mass action." This concept is based on the evidence available that enzymes and other proteins are in equilibrium with each other through a common pool of building materials or precursors. The pool of building materials includes all sources of carbon, nitrogen, vitamins and inorganic ions required. Various cell proteins as well as enzyme precursors are synthesized from and are in equilibrium with this pool.

In a subsequent paper experimental observations pertaining to an effect of dietary protein on the arginase activity of the liver of rats are described and related to the theory of enzyme adaptation by "mass action" (J. Mandelstam and J. Yudkin, *Biochem. J.* **51,** *681 (1952)*).

Animals utilized in these studies were maintained on a laboratory stock diet containing approximately 20 per cent protein until periods of experimental feeding were begun. Experimental diets were made up of purified food materials containing adequate quantities of all the essential nutrients. The only dietary constituent which was

varied was protein, which was provided as casein and composed 17, 33 and 67 per cent respectively of three different experimental diets. Food was provided ad libitum. Samples of liver were obtained at the time the animals were killed and the arginase activity determined by analysis of the urea produced by incubation of a homogenate with a substrate of arginine.

When groups of rats were placed on the experimental diets at an age of 5 weeks it was found that growth continued at the most rapid rate on a diet providing 33 per cent protein. Animals consuming a diet containing 17 per cent protein grew less rapidly, but achieved a body weight equal to that of those getting 33 per cent protein by the nineteenth week. When the diet contained 67 per cent protein growth was limited and proceeded at a slower rate.

Determination of the arginase activity of the livers of these animals demonstrated an increase with increasing quantities of protein in the diet, mean activities being 34.7, 41.6 and 59.2 units of arginase per milligram of liver for groups of rats consuming diets of 17, 33 and 67 per cent protein respectively. Arginase values represent milligrams of urea nitrogen produced in thirty minutes under the conditions of assay. The data are also presented in terms of arginase activity per milligram of hepatic nitrogen, per 100 g. body weight, and in the entire liver. By each means it is demonstrated that there is a significant increase in activity of this enzyme with greater intake of protein. The rate of increase is shown to be linear for enzyme concentrations related to weight of liver and to hepatic nitrogen. Since the absolute weight of the body and of the liver were less in the group of rats on the greatest protein intake, the total quantity of enzyme in the liver of these animals did not increase linearly.

Another experiment was undertaken in which the effects on hepatic arginase of feeding a diet containing 67 per cent protein in a group of young rats was compared with that in adult rats. These animals were 8 and 27 weeks of age respectively at the beginning of the experiment and were fed the high protein diet for periods of three weeks. In both groups of animals there was loss of weight in the first week of the experiment, but growth and gain in body weight occurred slowly thereafter. The concentration of enzyme per unit weight of liver, which was initially lower in the younger rats than in older animals, rose to even higher levels during adaptation to the high protein intake. When other animals after a similar period of adaptation were placed on the stock diet there was a decrease within four weeks almost to the initial level of arginase activity.

When arginase content was related to hepatic nitrogen it was found that the initial increase in enzyme in adult animals related to an increase in nitrogen in the liver. In young animals there was an immediate and great increase in enzyme. Similar findings were obtained when results were related to body weight, and with respect to the total amount of arginase in the liver.

These observations demonstrate that the arginase content of the liver increases with protein in the diet. Similarly, these values revert to lower levels when animals fed a very high protein diet are again placed on the stock diet containing only moderate amounts of protein. The authors, in attempting to explain these findings in terms of their previously stated theory of "mass action" enzyme adaptation, point out that the greater quantity of substrate afforded by the larger intake of protein requires a greater use of arginase in producing urea. Accordingly greater quantities of enzyme would be produced if more is in a combined form. The proportion of total enzyme in combined form might be increased because of greater formation of ammonia with more of this substance passing through the ar-

ginine cycle, or because of combination of the enzyme with substances derived directly from protein, *i.e.*, arginine, ornithine and lysine.

The authors pass lightly over the consideration that hepatic arginase may be increased because the quantity of many liver enzymes is increased with the greater amounts of protein available. In fact, other observers have found an increase in a variety of enzymes in liver with increased intake of food protein (*Nutrition Reviews* **9,** *103 (1951)*).

The linear increase in arginase in liver found in these experiments when greater quantities of protein were fed to rats is taken as evidence of conformity with quantitative deductions from the theory of enzyme adaptation. Whether or not one accepts these observations as partial proof for the validity of the authors' theory, one must view them with interest and consider them as adding significant new information concerning variation of the activity of an enzyme to varying quantities of a constituent in the diet.

PRESENT KNOWLEDGE OF LIPOIC ACID

Lipoic acid (thioctic acid; pyruvate oxidation factor; protogen; acetate replacement factor) is one of the newest biocatalysts that may come to belong to the family of B-vitamins. It has not yet been demonstrated as a dietary requirement for animal species, although other properties have been shown which are common to most B-vitamins (*e.g.*, recognized enzymatic function and a nutritional need by various types of microorganisms). For the present, it undoubtedly qualifies as an essential metabolite.

B. M. Guirard, E. E. Snell, and R. J. Williams (*Arch. Biochem.* **9,** *381 (1946)*) were early workers with lipoic acid, although nonspecific growth effects were noted earlier by V. C. Dewey and G. W. Kidder (*Biol. Bull.* **87,** *107 (1944)*; *Arch. Biochem.* **8,** *293 (1945)*). (These effects were later shown to be due to a mixture of lipoic acid, pyridoxal and copper ions; *see* E. L. R. Stokstad *et al.*, *Ibid.* **20,** *75 (1949)*). Guirard *et al.* observed that the stimulatory effect of acetate upon the growth of *Lactobacillus casei* could be replaced by small quantities of natural extracts such as liver, yeast, or dried grass juice. They concentrated the active material forty-four-fold from yeast. The following year, D. J. O'Kane and I. C. Gunsalus (*J. Bact.* **54,** *20 (1947)*) found that an unidentified factor from yeast (later called pyruvate oxidation factor; O'Kane and Gunsalus, *Ibid.* **56,** *499 (1948)*) was necessary in resting cells of *Streptococcus fecalis* for oxidation of pyruvate, or its anaerobic dismutation to lactate, acetate and carbon dioxide. Preparations were obtained that were approximately two hundred times as potent as the starting material (yeast extract).

Independent of these investigations, Stokstad *et al.* (*loc. cit.*) fractionated the natural supplements described by Dewey and Kidder for the nutrition of *Tetrahymena geleii*, and found that the necessary unknowns could be reduced to two. Both of these unknowns produced the same biologic response, and it was concluded that they were two forms of the same compound. They named the substance "protogen" because of its effect upon the protozöon. Later E. L. R. Stokstad, C. E. Hoffmann, and M. Belt (*Proc. Soc. Exp. Biol. Med.* **74,** *571 (1950)*) found that a strain of *Corynebacterium* also required protogen for growth. Finally, L. Kline and H. A. Barker (*J. Bact.* **60,** *349 (1950)*) described what appears to have been lipoic acid as a growth factor for *Butyribacterium rettgeri*. They concentrated the factor ap-

proximately two hundred-fold from yeast, and demonstrated the existence of at least three forms.

The probable identity of protogen, the acetate replacing factor, and the pyruvate oxidation factor was reported by E. E. Snell and H. P. Broquist (*Arch. Biochem.* **23,** *326 (1949)*), who showed a high degree of correlation to exist among the relative potencies of different concentrates for acetate replacement, pyruvate oxidation and *Tetrahymena* growth. The compound had been found to be fat-soluble, and because of this and its adsorption characteristics it was also thought to be similar to vitamin B_{13} (Kline and Barker, *loc. cit.*); however, the latter identity has not been confirmed experimentally.

The factor was isolated in pure form by L. J. Reed, B. G. DeBusk, I. C. Gunsalus, and C. S. Hornberger (*Science* **114,** *93 (1951)*). The term "lipoic acid" was also introduced at this point to describe the (fat-soluble) compound as it was obtained from liver after hydrolysis, possibly from ester or amide combination. The isolation procedure from yeast (Reed, DeBusk, P. M. Johnston, and M. E. Getzendaner, *J. Biol. Chem.* **192,** *851 (1951)*) disclosed the existence of five chromatographically distinct forms of the factor. Gunsalus, L. Struglia, and D. J. O'Kane (*Ibid.* **194,** *859 (1952)*) have separated these according to their solubility and acid strength, and it appears that they may be accounted for at least in part as natural conjugates of the vitamin. Only two forms appeared after hydrolysis (Reed *et al., Science, loc. cit.*), and one of these, a weak acid, was converted to a more polar form. A similar interconversion was reported in the isolation of protogen by E. L. Patterson *et al.* (*J. Am. Chem. Soc.* **73,** *5919 (1951)*); they observed a marked increase in water solubility, and designated the process protogen A → protogen B.

The potency of pure lipoic acid (called alpha-lipoic acid) was found to be 250,000 times that of yeast, based on the stimulation provided in the *S. fecalis* test for pyruvate oxidation. As a replacing agent for acetate in *L. casei*, lipoic acid was 15,000,000 times as potent; half-maximum growth was provided by 1.7×10^{-6} micrograms per milliliter of culture medium. This compound thus ranks with biotin, vitamin B_{12} and the folacin group as an extremely active biocatalyst.

Studies on the structure of the lipoic acids have been both intensive and numerous. The picture of the interrelationships among the various forms is rapidly emerging, although the important details are not yet all worked out. Reports during recent months have issued from the Lederle Laboratories and jointly from the Universities of Texas and Illinois and the Eli Lilly Laboratories. These all point to the lipoic acids as a family of dithioöctanoic acids and their derivatives. The synthesis of a pure compound with lipoic acid activity was accomplished by the Lederle group (M. W. Bullock *et al., J. Am. Chem. Soc.* **74,** *3455 (1952)*), who prepared the cyclic DL-6,8-dithioöctanoic acid, which they called 6-thioctic acid. It appeared to be roughly half as active in pyruvate oxidation as the isolated lipoic acid of Reed *et al.* Protogen B (beta-lipoic acid) may be presumed to be the corresponding (mono) sulfoxide (Bullock *et al., Ibid.* **74,** *1868 (1952)*).

The need for lipoic acid for the oxidation or dismutation of pyruvate places this catalyst in the same particular area of metabolism as thiamine and coenzyme A (Co A). The precise enzymatic role of lipoic acid has not yet been determined, although important indications are provided by the findings (L. J. Reed and B. G. DeBusk, *J. Am. Chem. Soc.* **74,** *3457, 3964 (1952)*) that "lipothiamide" (LT) and "lipothiamide pyrophosphate" (LTPP) are active in the oxidative decarboxylation of pyruvate. These compounds were prepared by reaction of alpha-lipoic acid or its acid chloride with thiamine and thiamine pyrophosphate, respectively. The products possessed chro-

matographic R_f values identical with those of two of the forms present in natural materials. Both gave negative thiochrome tests and positive azo tests, and the authors suggested that the carboxyl group of lipoic acid was conjugated with the pyrimidine amino group of thiamine. The test system used was a mutant strain of *Escherichia coli* that would not respond to either vitamin, alone or mixed together, but which grew luxuriantly on LT or LTPP. Soluble enzyme preparations from the mutant catalyzed the anaerobic dismutation of pyruvate or the oxidation of alpha-ketoglutarate only when LTPP was supplied (LT was inactive in the latter system). These reactions thus reveal a definite enzymatic role for lipoic acid in the oxidation of alpha-keto acids, as a molecular conjugate with thiamine, and point to LTPP as the actual coenzyme.

This scheme presented by Reed and DeBusk provides a plausible order of participation for LTPP and Co A, although it does not per se clarify the question of whether LTPP is needed for decarboxylation or dehydrogenation. In view of I. C. Gunsalus' observations (*In Press*) that cocarboxylase and *not* LTPP is needed for the conversion of pyruvate to acetoin (the latter compound is at the aldehyde level of oxidation), it would appear that LTPP may be involved in dehydrogenation, *i.e.*, at a very early stage in the breakdown of pyruvate. This is in line with the report by D. R. Sanadi, J. W. Littlefield, and R. M. Bock (*J. Biol. Chem.* **197,** *851 (1952)*) that pig heart alpha-ketoglutaric oxidase contains up to 6 moles lipoic acid and about 1 mole of cocarboxylase per mole of enzyme, but no DPN or Co A.

Such questions naturally arise as (a) the possible participation of lipoic acid in other types of systems, and (b) whether lipoic acid and thiamine may function independently. An independent role of cocarboxylase seems certain, not only in acetoin formation, as pointed out above, but also since the oxidation of pyruvate by ferricyanide or 2,6-dichlorophenolindophenol (Gunsalus, *loc. cit.*) is reported to be dependent upon cocarboxylase rather than lipoic acid. The question of participation of lipoic acid in other systems is indicated by its presence in animal alpha-ketoglutaric (Sanadi *et al.*, *loc. cit.*) and pyruvic oxidases (D. E. Green, *Science* **115,** *661 (1952)*).

The interest that the discovery of lipoic acid has engendered will doubtless continue for some time; in addition to further studies on the chemistry of the vitamin, the enzymic aspects of the problem deserve major attention. The finding in lipoic acid of a catalytically active thiol emphasizes again the importance of this class of compounds to biochemical systems. The realization that Co A and other thiols possess acetylating power in *in vitro* systems, and that the thiol bond may be of the "high-energy" variety (F. Lynen and E. Reichert, *Angew. Chemie* **63,** *47 (1951)*), focuses additional interest upon the lipoic acid system as a possible participant in energy conservation and transport.

AMINO ACID IMBALANCE

Once the so-called essential amino acids were identified, it soon was shown that the nonessential amino acids exerted a distinct effect in protein metabolism as shown by studies on the growth of rats with purified experimental rations. Closely associated with this point of view is the subject of amino acid imbalance: certain amino acids or proteins appear to accentuate the demand of the organism for other amino acids. Thus knowledge of the now well-known relationship between tryptophan and niacin grew out of studies (W. A. Krehl, P. S. Sarma, and C. A. Elvehjem, *J. Biol. Chem.* **162,**

403 (1946)) showing that the need of the growing animal for tryptophan was increased when corn grits, or gelatin, or hydrolyzed fibrin was added to a low-casein or niacin-poor ration. Other studies showed that even hydrolyzed casein added to a niacin-low diet interfered with the utilization of tryptophan. More specifically, it was demonstrated (L. V. Hankes, L. M. Henderson, W. L. Brickson, and Elvehjem, *Ibid.* **174,** *873 (1948)*) that DL-threonine and DL-phenylalanine in amounts equivalent to 2 per cent of hydrolyzed casein interfere with the metabolic processes involved in the tryptophan-niacin relationship. Threonine inhibited growth, in this study, whether administered parenterally or by mouth.

An approach to the explanation of the threonine-tryptophan antagonism has been made in further growth studies (K. Ebisuzaki, J. N. Williams, and C. A. Elvehjem, *J. Biol. Chem.* **198,** *63 (1952)*). Young rats were fed the following purified rations: a complete 9 per cent casein-sucrose diet alone and with 0.078 per cent DL-threonine, and a complete diet containing an amino acid mixture simulating 9 per cent casein, alone and with 0.078 per cent added DL-threonine. Six rats constituted each of the four groups and the experiments were terminated after thirty-six days. The average increase in body weight of the casein-alone group was 11 g. per rat per week whereas when 0.078 per cent threonine had been added to the ration the growth was represented by a gain of 1 g. per week.

The decision that this difference in response in body weight was due to a deficiency in tryptophan rests on the similarity of appearance of these animals to those described in an earlier paper (R. L. Lyman and C. A. Elvehjem, *J. Nutrition* **45,** *101 (1951)*), the typical symptoms being "a porphyrin exudate around the nose and eyes, unkempt hair and emaciated appearance" and on the known curative effect of tryptophan and niacin (L. V. Hankes, L. M. Henderson, and Elvehjem, *J. Biol. Chem.* **180,** *1027 (1949)*). When threonine was added to the amino acid mixture the increase in body weight was 14 g. per week whereas that for the amino acid mixture without the added threonine was 15 g. per week. It is plain that under these experimental conditions added threonine did not inhibit growth when the amino acid mixture was fed as the main source of dietary nitrogen. Essentially the same results were secured when 0.156 g. DL-threonine was used in the tests.

Inasmuch as threonine interferes with the utilization of tryptophan when intact protein is fed but not when the analogous amino acid mixture is ingested, it appears that threonine interferes with some phase of the digestive process in the alimentary tract. That this is the explanation is suggested by the observation that when rats are forcibly fed a 9 per cent casein diet plus 0.078 per cent DL-threonine, the food is slowly digested and absorbed, causing a marked bloating of the stomach (S. S. Shahinian *et al., Proc. Soc. Exp. Biol. Med.* **80,** *146 (1952)*). However, the fact that the inhibiting action of threonine can be exerted when the amino acid is administered intraperitoneally suggests that it may interfere with the elaboration of one or more of the digestive enzymes.

NOTES

American Board of Nutrition

The American Board of Nutrition will meet in April 1953 to consider applications of candidates for certification as Specialists in Human Nutrition. Completed applications should be in the office of the Secretary not later than March 15. Candidates who are deemed eligible for examination will be notified of the date, place and procedure of the examination, after the meeting of the Board. Application forms may be obtained from the Secretary, Otto A. Bessey, School of Medicine, The University of Texas, Galveston, Texas.

The complete list of Specialists in Human Nutrition, certified by the Board as of current date, may be found in the February 1953 issue of the *Journal of Clinical Nutrition.*

Secular Changes in Growth

Published data of heights and weights of schoolboys in London, England, and Glasgow, Scotland, have recently been carefully analyzed by J. B. de V. Weir of the University of Glasgow (*Brit. J. Nutrition* **6,** *19 (1952)*). The changes in height and weight between the ages of 6 and 14 years were investigated in terms of age of attaining a given stature and also in terms of rate of growth.

In forty years the average height of London and Glasgow schoolboys has increased about 3 inches and the average weight about 9 pounds. The presently available data indicate that these increases in height and weight are not maintained into adult life. It is thus debatable, according to Weir, whether or not there is any increase in the mean adult maximum height or weight. If any real increase in the adult has occurred, it is apparently much less than the increases that have occurred in children.

These analyses have led Weir to the conclusion that today British children are growing faster and thus achieve adult stature at an earlier age than children of previous generations.

Since rate of growth is very widely used as an index of nutritional status, these data would suggest that improved nutrition may be largely responsible for the accelerated growth rate of British children in the present generation.

A New Fluorometric Determination of Thiamine

Few analytic procedures are more important to the nutritionist than the determination of thiamine. So far colorimetric and fluorometric methods used have been found to suffer often from a lack of sufficient sensitivity or specificity. A new method has recently appeared (A. E. Teeri, *J. Biol. Chem.* **196,** *547 (1952)*) which is claimed to present a greater sensitivity and specificity than other chemical procedures hitherto used. It is based on the fact that thiamine in a buffered water-alcohol solution reacts with cyanogen bromide to form a highly fluorescent compound. Of a number of substances tried, only pyridoxal in high concentrations was found to cause fluorescence under the conditions of determination. Promptness in measuring the fluorescence after the reagents have been mixed minimizes this cause of inaccuracy. Data are presented which suggest excellent agreement between the new chemical method and a classic bacteriologic procedure.

New Journal

The American Chemical Society, 1155 16th Street, N.W., Washington 6, D. C., announces the publication of the journal of *Agricultural and Food Chemistry,* edited by Walter J. Murphy. Publication will be biweekly and is to begin in April 1953. Subscription rates are $6.00 per year, and subscription applications should be sent to the American Chemical Society at the above address.

Breakfast for Teen-Agers

Under a cooperative regional research project on nutritional status in the northeastern states, the Maine Agricultural Experiment Station has published results of a study on the nutritive value of breakfasts of Maine teen-age children and the contribution of the breakfasts to the day's food intake (M. M. Clayton, *Maine Agr. Expt. Station Bull.* **495** (*1951*)).

The subjects of the study were 27 girls and 30 boys of junior high school age randomly selected from 780 children in three Maine communities. Food consumption and nutritive value data were calculated from the food intake records kept by the children for a seven-day period.

Only a few of the children ate breakfasts which supplied as much as one-third of the daily allowance of the various nutrients as recommended by the National Research Council (*National Research Council, Reprint and Circular Series No. 129, Washington, D. C.* (*1948*)). Less than half of the children received one-fourth or more of the recommended daily intake of calories, protein, iron, vitamin A and niacin in their breakfasts. Forty per cent of the children had less than 15 per cent of the recommended daily allowance for vitamin C in their breakfasts. On the average, vitamin C-rich foods, such as citrus fruits and juice and tomato juice, were consumed for breakfast about three mornings in seven.

Diets for the whole day were low in calcium and vitamin C; less than 60 per cent of the children were consuming as much as 80 per cent of the recommended daily allowances of these nutrients.

The publication includes a listing of breakfast menus that are designed to supply 25 per cent of the recommended daily allowances of the various nutrients.

The Determination of Vitamin B_{12}

A chemical method for the determination of vitamin B_{12} has been reported by G. O. Rudkin and R. J. Taylor (*Anal. Chem.* **24,** *1155* (*1952*)). The method is based on the difference between the visible spectrum of vitamin B_{12} and its dicyanide complex. The latter compound has an absorption maximum at 582 millimicrons which the free vitamin does not possess. Ten separate analyses of each of 3 samples indicated a standard deviation of 5 per cent when the above method was used. The same workers note that the standard deviation for the *Lactobacillus leichmannii* microbiologic method for vitamin B_{12} was 15 per cent. A greater degree of reliability for the proposed chemical method over the much used microbiologic method is thus indicated. The method is proposed particularly for vitamin B_{12} determination in fermentation broths and crude concentrates and has a reliability not hitherto found with other published methods (*Nutrition Reviews* **9,** *251* (*1951*)).

Evaluation of Foods

In a recent communication to the editor of the *Journal of the American Medical Association* (**149,** *1333* (*1952*)) E. F. Kohman discusses a method for evaluation of foods.

The author presents a table of the ratio of daily requirements of nutrients to daily requirements of calories in various foods. For this comparative evaluation of foods the values for a 154 lb. (70 kg.) sedentary man and a moderately active 123 lb. (56 kg.) woman are used since they are alike for most of the nutrients. Figures are given for various canned vegetables and fruits, canned fish and the noncanned staple foods, bread, milk, beef and eggs.

Since the data presented are based on chemical and microbiologic technics, they reveal nothing on availability of these nutrients. If data on availability were used, these figures would assume greater significance.